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SPECIFICITY OF HEAVY METALS DISTRIBUTION IN *ATROPA BELLADONNA* PLANTS

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Aim. To investigate the content of heavy metals (HM) in the seeds and roots of *Atropa belladonna* plants. To identify possible changes in their concentrations over the years of growing these plants. **Methods.** Field and spectrometric method, methods, common for agriculture, plant growing and statistics. **Results.** The content of heavy metals (HM) in the seeds and roots of belladonna plants was determined. It was found that plant roots accumulate them much more compared to the seeds. The concentration of such HM as barium, cadmium, copper, iron, manganese, nickel, potassium, sodium, strontium, and zinc in the plants exceeds the maximum permissible level, which testifies to partial soil contamination in the region. **Conclusions.** As there are no data about maximum permissible ratios for the content of important chemical elements in the raw plant material, specified in normative and regulatory documents, scientists have to launch complex investigations, involving specialists of relevant fields to identify the mentioned gradation. It was determined that the main part of HM is accumulated in the roots of belladonna plants, whereas their content in the seeds is several times smaller or equals the value under the sensitivity threshold of analytical devices. This fact testifies to the prospects of growing the mentioned medicinal herb in Kyiv region for further industrial processing. Chemical elements, which belong to compounds of lanthanides and actinides, are almost not accumulated in belladonna plants.

Keywords: biological raw materials, maximum permissible ratio, chemical elements.

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INTRODUCTION

A great number of useful plants, including medicinal, potherbs, essential oil and other herbs, used by people for centuries, grow on the lands of Ukraine. However, the flora, established in the process of plant kingdom evolution, is petering out due to its indiscriminate exploitation, excessive tillage of lands, construction, building of industrial objects, etc. Natural resources of useful plants have been insufficient for a long time. Due to this fact, since 1930's Ukraine has been growing some medicinal, potherbs and technical herbs. At present their number has increased. Among these plants, *Atropa belladonna* takes a prominent place of high industrial relevance.

Belladonna (*Atropa belladonna* L.) belongs to medicinal herbs with a wide spectrum of pharmacological activity, used in scientific medicine for a long time. The impact of belladonna on the organism is mainly determined by the effect of atropine and scopolamine [1, 2]. Belladonna preparations are widely used in medical practice as anti-inflammatory, anti-spasmatic means, and pain relievers for gastric and duodenal ulcers, cholecystitis, gallstone disease, renal colics, in ophthalmologic practice – to dilute the pupil and to paralyze the accommodation, as well as for other diseases, related to vegetative nervous system disorders [3].

To provide the domestic medical production with raw materials of belladonna, the industrial production of the latter was previously concentrated in the Crimea and in the Transcarpathian region. Belladonna is a macrothermophyte, its growing is limited by winter conditions [4, 5]. At present the domestic pharmaceutical industry is not sufficiently provided with belladonna plant material. Therefore, there is a need of expanding the area of its growing, involving regions of Ukraine, not traditional for this plant, and the elaboration of new elements of growing techniques. As it is important to determine both the content of biologically active substances (BAS) and the ratio of heavy metals (HM) in the biological raw materials and other components of medicinal plants, the monitoring of its level is of great relevance. This information may be used for the purpose of timely formation of the system of measures, aimed at the optimization of the content of both BAS, required in pharmacology, and chemical elements to maximum permissible ratios.

The scientists of the Institute of Horticulture (IH), NAAS of Ukraine, are working at the investigation, elaboration, and improvement of the elements of the technology of growing *Atropa belladonna* which would correspond to the requirements of obtaining quality pharmacological raw materials.

The formation and accumulation of BAS by medicinal herbs is a dynamic process, changing over the plant ontogenesis, which depends on many environmental agents, including anthropogenic ones. In addition, a number of dangerous pollutants – highly toxic HM – are capable of penetrating the biological circulation and getting accumulated in the human organism. Thus, at present it is urgent to study the specificities of HM migration in the system of "soil–medicinal herb–period of keeping biological raw material" as well as to investigate their introduction into the biological circulation and accumulation in the human organism.

The aim of our study was to determine HM content in the seeds and roots of *Atropa belladonna* and to define possible changes in their concentrations over the years of growing these plants. These data will be used to elaborate relevant sanitary-hygienic and economic organizational measures, ensuring the required level of ecologic safety and rational use of the obtained yield of *Atropa belladonna* plants.

MATERIALS AND METHODS

Field studies were conducted in permanent study conditions at the Laboratory of ornamental, medicinal and essential oil plants of IH NAAS, laboratory studies – at the Institute of Physiology and Genetics of NAS of Ukraine in 2014–2016. The scheme of the experiment envisaged the rotations of herbs in short field rotations and included the following variants: purple echinacea (*Echinacea purpurea*) – wild chamomile (*Matricaria recutita*) – clary sage (*Salvia sclarea* L.). The process of growing these plants does not require any fertilizers or the use of pesticides on the fields. The field experiments were scheduled in three variants with the total area of the seedling plot of 20 sq.m., the registration plot – 10 sq.m., with three repeats. The starters were transferred to the field in early May in the phase of two actual leaves. The variants of planting schemes were as follows: 1) 60 × 60; 2) 60 × 50; 3) 50 × 50 sq.cm.

The crop tending involved manual weeding in the rows as required.

The plants were watered during the vegetation period only in case of long-term absence of rains, mainly in July of 2014 and 2015.

The migration of HM is considerably dependent on the soil type, its mechanical composition, aqueous-physical and agrochemical properties. Thus, a detailed analysis of the soil of experimental plots was performed in the Agrochemistry laboratory of IH NAAS. The content of humus in the arable layer (0−40 cm) was 2.3 %, that of easily hydrolyzed nitrogen (according to Turin and Kononova) − 78.4−98.0 mg/kg, forms of mobile phosphorus (according to Kirsanov) – 93.2−180.9 mg/kg, exchange potassium (according to Kirsanov) – 106.1−202.8 mg/kg. The reaction of their soil solution is acid (pH level of 5.3–5.8 and 5.5–6.1, respectively).

The content of HM in the roots and seeds was analyzed at the Institute of Physiology and Genetics of NAS of Ukraine. The elemental composition in the experimental samples was determined by ICP-MS method using the emission mass-spectrometer Agilent 7700×. The samples were dried until the dry mass and incinerated in the nitric acid (ACS) using the microwave system of sample preparation Milestone Start D. The volume of the obtained extract was adjusted to 50 ml using class 1 water (18 megaohm), purified at the Scholar-UV Nex Up 1000 system (Human Corporation, Korea).

The results of the experiments were statistically processed by standard methods [6, 7] and Excel program, and subsequent processing of the data obtained, using the professional software package for statistical analysis Statistica 8.0.

RESULTS AND DISCUSSION

In recent years the anthropogenic impact on natural complexes has considerably increased in the world. In Ukraine the impact on environment is lower than the average global index, however, when the matter relates to herbs, especially medicinal herbs, the quality of their raw material requires constant control and subsequent optimization of the elements of growing techniques.

The results of the analysis of HM content accumulation in the seeds and roots of *Atropa belladonna* on average for three years of growing (2014–2016), defined in [8], are presented in Tables 1 and 2.

The analysis of the data in Table 1 regarding the availability and concentration of chemical elements in the seeds and roots of *Atropa belladonna* demonstrated that the root system accumulates much more chemical substances compared to the seeds. Over three vegetation periods on average a great difference in HM accumulation was noted for the following substances: aluminum – over 40 times more in the roots compared to the seeds, barium – over 10 times more, calcium – over 6 times more, iron – over 4 times more, sodium – over 2 times more, strontium – over 8 times more.

Most mentioned elements exceed the maximum permissible ratio (MPR) in the roots, where in the seeds their content is in the normal range. For instance, the concentration of aluminum in the roots of belladonna plants usually exceeds MPR almost 15 times. This metal is ranked the third (after oxygen and silicon) wide-spread element in nature. If the concentration of aluminum in the soil solution is close to the phytotoxicity threshold, there is evident inhibition of most plants, while its absence or insufficient amount results in chlorosis [9].

The content of barium in the roots of investigated belladonna plants is almost 30 times exceeding the

Table 1. The accumulation of heavy metals by medicinal plants, 2014–2016

Element	Concentration of heavy metals, mg/kg		MPR* of heavy metals in plants, mg/kg
	Seeds	Roots	
Aluminum	6.85 ± 0.01	297.36 ± 0.004	20
Barium	2.96 ± 0.007	20.77 ± 0.003	0.7
Beryllium	< 0.00	0.12 ± 0.03	0.0002
Cadmium	0.11 ± 0.008	0.14 ± 0.01	0.03
Calcium	104.68 ± 0.005	678.77 ± 0.004	n.d.**
Cesium	0.006 ± 0.022	0.066 ± 0.009	n.d.
Chromium	0.046 ± 0.01	4.77 ± 0.003	0.2
Cobalt	0.028 ± 0.02	0.23 ± 0.005	0.1
Copper	11.61 ± 0.001	7.44 ± 0.002	5.0
Iron	51.55	241.05 ± 0.001	0.3
Lead	< 0.00	0.37 ± 0.004	0.3
Lithium	< 0.00	< 0.00	0.03
Magnesium	2338.28 ± 0.004	2036.54 ± 0.014	n.d.
Manganese	18.71 ± 0.003	17.56 ± 0.003	2.1
Nickel	1.13 ± 0.002	2.89 ± 0.003	0.5
Potassium	4629.73 ± 0.006	7928.79 ± 0.002	10.0
Rubidium	2.11 ± 0.005	0.05 ± 0.124	n.d.
Silver	0.006 ± 0.005	0.04 ± 0.021	0.5
Sodium	99.90 ± 0.001	241.29 ± 0.002	20
Strontium	3.48 ± 0.005	24.08 ± 0.004	1.0
Thallium	0.004 ± 0.003	0.08 ± 0.015	n.d.
Zinc	50.58 ± 0.001	15.03 ± 0.003	10.0
Bor	7.90 ± 0.005	13.33 ± 0.005	n.d.
Gallium	0.09 ± 0.009	0.63 ± 0.005	n.d.
Arsenic	0.006 ± 0.004	0.14 ± 0.01	0.2
Selenium	0.16 ± 0.004	0.10 ± 0.004	n.d.
Phosphorus	4580.67 ± 0.004	1397.15 ± 0.001	n.d.

Note. *Maximum permissible ratio; **no data.

MPR (the permissible ratio for the medicinal plants is 0.7 mg/kg). The impact of barium on the human organism has not been studied in fine detail. Nevertheless, at present scientists refer it to toxic ultramicroelements [10].

The concentration of iron compounds in the biological raw material of the plant exceeded the MPR considerably: over three years of growing on average it was over 50 mg/kg in the seeds and over 240 mg/kg – in the roots of plants.

Regardless the fact that the content of iron in a person of 70 kg does not exceed 5 g and the daily requirement is 10–15 mg, it plays a special role in the activity of the organism.

Iron is of special relevance as the impact of the secretory system does not cover it. Iron concentration is regulated by its consumption only, not by its release. About 65 % of all iron in the organism of the adult person is in hemoglobin and myoglobin, more than a half of what is left is kept in special proteins (ferritin and hemosiderin) and only a very small amount is in different enzymes and transportation systems [11].

The imbalance of iron in the organism promotes excessive accumulation of the toxicity of valuable metals in the central nervous system. Thus, it is important to control its accumulation in the plants, allocated for the production of medicines.

The concentration of strontium compounds in the samples of belladonna seeds exceeds the MPR over three-fold, and in the roots – over 24 times (see Table 1).

The accumulation of strontium by different organisms depends not only on their type and specificities, but also on the ratio of this metal in the environment with other elements, mainly with calcium and phosphorus, and also on the adaption of organisms to specific geochemical environment.

Living organisms receive strontium with water and food. Strontium is mainly absorbed with small intestines, and released with large intestines. The main strontium depot in the organism is the bone tissue, the ashes of which contain about 0.02 % of this element (compared to about 0.0005 % in other tissues). The increased content of strontium in the organism results in the fragility of bones, rickets, and other diseases. The manifestation of Kashin-Bek disease is observed in the biogeochemical provinces, rich in strontium (a number of regions in the Central and Eastern Asia, North Europe, and others) [12].

However, not all the HMs are predominantly accumulated in the roots of *Atropa belladonna* plants. Such chemical elements, as cobalt, copper, magnesium, manganese, rubidium, zinc, phosphorus are much more accumulated in the seeds compared to the root system. While the content of cobalt, copper, and manganese is

Table 2. The accumulation of heavy metals by medicinal plants

Element	Concentration of heavy metals, mg/kg	
	Seeds	Roots
	Lanthanides	
Praseodymium	< 0.00	0.07 ± 0.010
Samarium	< 0.00	< 0.00
Gadolinium	< 0.00	0.08 ± 0.008
Dysprosium	< 0.00	0.04 ± 0.016
Holmium	< 0.00	0.01 ± 0.004
Erbium	< 0.00	0.02 ± 0.002
Thulium	< 0.00	0.008 ± 0.053
Lutecium	< 0.00	0.009 ± 0.001
Cerium	0.012 ± 0.006	0.56 ± 0.005
Lanthanum	0.005 ± 0.014	0.28 ± 0.005
	Actinides	
Thorium	< 0.00	0.13 ± 0.008
Uranium	0.003 ± 0.001	0.03 ± 0.002

insignificantly exceeding their ratio in the seeds, the content of magnesium is 302 mg/kg higher, that of rubidium – 2.06 mg/kg higher, zinc – over 35 mg/kg higher, phosphorus – over 3,000 mg/kg higher. It is difficult to explain such a difference in HM accumulation by different parts of the same plant. The experiments are underway, and the analysis of their results may allow determining the mechanism, via which plants accumulate specific chemical elements.

As the quantitative index of the availability of phosphorus in the seeds of belladonna is very high, it is reasonable to discuss the role of this element for the human organism. Phosphorus is the element of energetics and mind, a constituent of highly energetic compounds, fulfilling the function of fuel, the universal energy carrier. Along with calcium it is responsible for firmness and stability of the bone tissue, compounds of phosphorus participate in the exchange processes of fats, proteins and carbohydrates. Our daily requirement for phosphorus is from 1 to 4.6 mg. If the concentration of phosphorus in human blood is over 1.8 mmol/l, it testifies to the excess (hyperphosphatemia) of this mineral in the organism. The excessive amount of this substance has negative effect on the nervous system, impairs the absorption of calcium in the intestines, inhibits the formation of the active form of vitamin D [13]. Therefore, it is reasonable to assert (even in conditions of unavailability of any information about MPR) that in Kyiv region the concentration of phosphorus, which is 4580.67 mg/kg in the seeds and 1397.15 mg/kg in the roots of belladonna plants, is rather high. It is necessary to elaborate the elements of the technology of decreasing the ratio of this element in the medicinal biological raw materials.

Similar to other microelements and vitamins, zinc is also important for the organism. It stimulates the immunity and normalizes the endocrine profile. Its daily requirement for an adult person is 15 mg [14].

A smaller ratio of zinc compounds, compared to phosphorus, was identified in the samples of the investigated medicinal herb. However, MPR, defined for this chemical element, indicates the following considerable excess in zinc ratio compared to the permissible level over three years of growing: in the seeds – by 40–58 mg/kg, in the roots – by 5.03 mg/kg.

Over three years of growing, the excess of MPR for cadmium compounds in the biological raw materials of *Atropa belladonna* was 0.08 mg/kg in the seeds, and 0.11 mg/kg – in the roots of plants.

According to the scientific literature, the content of cadmium in plants is 0.001 % (per dry substance). In the zones of increased content of cadmium in soil, there is notable 20–30-fold increase in its concentration in the aboveground part of the plants compared to the plants of non-contaminated territories. Contaminated plants may contain up to 400 mg/kg of cadmium and more. Contrary to other mineral elements (except for zinc), cadmium may get accumulated in the generative organs in relatively large amounts. On average, its content in the seeds of plants, growing at the contaminated territories, increases up to 4 mg/kg (in the seeds of plants from “clean” territories – 0.2 mg/kg of cadmium) [15].

The results of the analysis testify that in the biological raw materials of *Atropa belladonna*, the plants of which vegetated in the soils of the Kyiv region, the concentration of cadmium was slightly exceeding the maximum permissible ratio, but it should be noted that the seeds accumulated 0.3 mg/kg less of this element compared to the root system.

Taking into consideration the fact that the biological raw material of belladonna is mainly subject to processing, which promotes its purification from heavy metals greatly, the excess of cadmium in the soils of the region is not a problem for growing this medicinal herb.

In the experiments the vegetative objects are characterized by rather high content of manganese, which exceeds MPR in the seeds by 11 %, and that in the roots of belladonna – by 12 %.

Manganese takes active part in the exchange of substances, in the oxidative processes, in the reduction of nitrites during the photosynthesis, it also improves physiological processes. The antagonism between manganese and other elements, iron in particular, was established. Manganese is a constituent of enzymes, enhancing their activity. Its average content in plants is 0.001 % (1 mg/kg of dry mass).

This element is received by the plant during the whole vegetative period. Both the excess and shortage of manganese have negative impact on plants [16]. The results of the analysis demonstrated that on average over three years of investigations the seeds and roots of plants were almost equal in accumulating manganese, the content of which was somewhat exceeding MPR, similar to previously described chemical elements. It may be related to the increase in anthropogenic load on the experimental plot, which is located only one kilometer away from the road with active traffic of vehicles.

In order to have better understanding of the course of HM migration and accumulation processes in the contaminated soils in the region of growing plants, further studies are scheduled for plots, remote from the highways, which will provide for better study of the accumulation of chemical elements in the plants.

The content of such elements as beryllium, lithium, and lead was close to the value under the sensitivity threshold of analytical devices.

The compounds of lanthanides and actinides are referred to the group of rare earth metals. Their maximum permissible ratios in medicinal plants are not mentioned in the specialized literature. However, there was a study of their availability in belladonna plants. The results of the analysis are presented in Table 2. These results proved to be remarkable, as one may state a conclusion that different parts of belladonna plants usually accumulated chemical elements, belonging to lanthanides and actinides, in a different way. Most of them are present in the roots of plants, whereas the seeds accumulate the value under the sensitivity threshold of analytical devices.

According to the results of three years of investigations, such chemical element as uranium, which belongs to actinides, gets accumulated in the biological raw materials of *Atropa belladonna* both in the seeds and in the roots. However, its content is insignificant. Taking into consideration the fact that uranium and its compounds are radioactively and chemically toxic for humans, it is required to monitor the intake of this element in the plants, especially medicinal ones. The toxic effect of uranium is conditioned by its chemical properties and depends on solubility. The contamination with uranium and its compounds is possible in the enterprises of mining and processing uranium raw materials and other industrial objects, where it is used in the technological process. Once penetrating the organism, uranium affects all the organs and tissues, as it is a general cellular poison. The signs of poisoning are usually manifested in the damage of kidneys, liver, and gastrointestinal tract [17].

Finally it should be noted that HM are natural components of the earth's crust, as a rule they are present in all the ecologic matrices. Nevertheless, HM concentration increased several times due to the anthropogenic activity in several ecosystems. The contamination of environment with HM is getting global, which is mainly related to their toxicological risks for human health. At the same time, HM are a relevant component of living organisms, they manifest their toxicity only in high concentrations.

It was established that there is partial contamination of soils in the Kyiv region with specific chemical elements, which are referred to HM. Therefore, the detoxication measures are required to grow agricultural crops in general and medicinal herbs in particular on such soils. Chemical detoxication of HM in the soil envisages the introduction of ameliorants – compounds, the effect of which is aimed at transforming the pollutants – into less toxic forms. These are complex studies, envisaging the participation of specialists of adjacent professions, but these investigations are really necessary.

CONCLUSIONS

Most chemical elements, which are referred to heavy metals, have a higher accumulation level in the seeds of *Atropa belladonna*. However, such elements as Cu, Mg, Mn, Rb, Zn, P, are considerably more accumulated in the roots of plants compared to the seeds.

The concentration of such chemical elements as Be and Pb in the seeds of belladonna is close to the value under the sensitivity threshold of the analytical devices, whereas in the roots it exceeds MPR.

Chemical elements, which belong to compounds of lanthanides and actinides, are almost not accumulated in belladonna plants, grown in the Kyiv region.

Further efforts of scientists should be concentrated on the complex solution of the problem, namely, the combination of measures in chemical detoxication (introduction of ameliorants) with the elements of novel technology of growing *Atropa belladonna* plants.

Специфіка розподілу важких металів у рослинах беладони звичайної

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Мета. Дослідити вміст важких металів (ВМ) у насінні та коренях беладони звичайної. Виявити можливі зміни їхніх концентрацій за роками вирощування рослин культури. **Методи.** Польовий, спектрометричний, загальноприйняті у землеробстві, рослинництві і статистиці методики. **Результати.** Визначено вміст ВМ у насінні та коренях беладони звичайної. Виявлено, що ко-

рені рослини акумулюють їх набагато більше порівняно з насінням. Концентрація в рослинах таких ВМ, як барій, кадмій, мідь, залізо, марганець, нікель, калій, натрій, стронцій і цинк, перевищує гранично допустимий рівень, що свідчить про часткове забруднення ґрунтів регіону. **Висновки.** Відсутність у нормативно-правових документах відомостей про гранично допустимі коефіцієнти відносно вмісту важливих хімічних елементів у біосировині лікарських рослин зобов'язує науковців розпочати комплексні дослідження із залученням фахівців відповідних профілів для встановлення зазначеної градації. З'ясовано, що основна частина ВМ акумулюється в коренях рослин беладони звичайної, тоді як у насінні їхній вміст знижується в рази або до величини, меншої за поріг чутливості аналітичних приладів. Це свідчить про перспективність вирощування згаданої лікарської рослини в Київській області для промислової переробки. Хімічні елементи, які належать до сполук лантаноїдів і актиноїдів, майже не накопичуються в рослинах беладони звичайної.

Ключові слова: біосировина, гранично допустимий коефіцієнт, хімічні елементи.

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